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### **ABSTRACT**

Introduction: As mindfulness is considered as a predictor of day-to-day self-regulated behavior and adaptability to stressful events, the present study investigated the psychophysiological and cognitive responses induced by a military training for escaping to a submarine simulator according to the submariners' mindfulness level. Methods: Sympathovagal balance, salivary cortisol, mindfulness level, mood and sleep perception as assessed by questionnaires, and short-term and declarative memory were assessed in submariners on shore and at sea before the escape (Baseline), immediately after the escape (Escape) and two hours after it (Recovery). Results: Compared to the simulation exercise, the naturalistic condition induced (i) higher degradations in mood, including vigor (p < 0.05), tension (p < .05), and easiness to go to sleep (p < 0.05), (ii) higher salivary cortisol values ( $6.33 \pm 3.9 \text{ nmol/L}$  on shore and  $13.38 \pm 7.5 \text{ nmol/L}$  at sea, p < 0.05), and (iii) impairment in the declarative memory ( $9.3\pm 1.4$  free recalls for a maximum of 12 on shore and  $7.5\pm 2.4$  at sea). On shore, responses differed according to the subject's past submarine accident, with lower vagal recovery (p < 0.05), higher cortisol responses across the exercise time (p < 0.05), and lower declarative memory performances after the escape (p < 0.05). Submariners high in mindfulness score exhibited both lower stress reaction and lower cognitive degradation. Conclusion: Psychophysiological and cognitive changes induced by military exercises are influenced by realistic conditions and by subject's mindfulness level.

### 1.0 INTRODUCTION

Exposures to acute stress are inevitable components of military life. Thus, studies in the military context are of considerable interest to researchers investigating stress mechanisms. Over the years, stress responses and cognitive degradations to diverse military contexts have largely been documented using scenarios designed to simulate stressful situations [1]. These studies activate biological stress reactions sometime at intense levels.

The response to acute stress involves allostasis as the process of achieving homeostasis through physiological and behavioural changes faced to perceived demand [2,3]. The main physiological response involves the Autonomic Nervous System (ANS), which controls the release of adrenalin from the sympathetic-adrenal-medullary system, and the Hypothalamo-Pituitary-Adrenal (HPA) axis which products cortisol. Stress is thought adaptative as it promotes emergency coping under an energetic crisis [fight or flight response). However, some data highlight its negative influence on cognition [1,2,3,4]. While it is unclear which aspects on cognition could be impaired by an acute stressor and to what extent, an enhanced stress reaction has been associated to impairments in declarative memory and preserved short term memory [4].

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Furthermore, Mindfulness, described as a non-elaborative, non judgmental present-centred awareness in which each thought, feeling or sensation that arises in the attentional field is acknowledged and accepted as it is [5,6,7], appears as an attribute of conscientiousness long believed to promote well-being [8]. Indeed, mindfulness training is related to positive psychological and physiological outcomes [9,10]. A high level of mindfulness increases willingness to tolerate uncomfortable emotions and sensations [11,12] and emotional acceptance [13,14,6]. It also decreases the impact of negative emotional events and reduces time to recover [5]. Mindfulness is therefore applied in the treatment of various anxiety disorders [15,16]. Thus, it appears as a predictor of day-to-day self-regulated behavior and adaptability to stressful events. Unfortunately, data on the relationship between mindfulness level and psychobiological and cognitive stress-responses are scarce.

Interestingly, a significant warrior culture in history has used mindfulness to focus their mind and sharpen their skills as fighting men: the Samurais. They practiced mindfulness to bring order to their mind and cultivate their awareness. The belief was that practicing moment-to-moment awareness would increase their prowess in battle by reducing or eliminating fear and experiencing a battle as it's actually happening instead of how their mind may want it to turn out. Having an uncluttered mind and not being emotionally attached to an outcome also allowed the Samurais to act more spontaneously in battle and in politics as they moved into leadership positions.

The present study, based on an exercise for escaping to a submarine simulator, was therefore initiated to investigate the subject psychobiological reactions when escaping according to their mindfulness level. Stress reactions were evaluated using physiological (*i.e.*, cortisol level, sympathetic activation) and behavioral (*i.e.*, cognitive and emotional performances) markers at various times from stressor exposure. It was hypothesised that quality and intensity of both physiological and cognitive responses were modified by subject's mindfulness level.

#### 2.0 MATERIELS AND METHODS

### 2.1 Subjects

Thirteen healthy males (mean age 30.8 years, SD 4.6) were recruited via opportunity sampling from submarines attending simulated submarine escape training at the French Training Institute to Submariner Safety in Brest. They had served  $10.7\pm5.2$  years on active duty. Their educational background varied from 1 year (*i.e.*, submarine school) to 4 years (*i.e.*, more specialized technical courses). They had submarine simulator training experience (number of training:  $3\pm1.5$ ). All volunteer individuals gave written informed consent before participation. The study was approved by the ethics committee of the French Military Health Service.

# 2.2 Operational and experimental procedures

The Submarine headquarters, based at Brest, organized the escape training in the submarine simulator in October 2007. The escape rate was of one submariner every twenty minutes to be as close as possible from what would be done in case of real emergency in a sunk submarine.

The training procedure consisted in one theoretical training session including the psychological questionnaires and cognitive tasks training following by the escape training exercise which took place in the submarine airlock simulator (Simulation). For all subjects, the escape exercise was carried out between 15h30 and 18h00 in order to control circadian variation. A 12 h-night urines were collected the night before the escape and

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mood and sleep questionnaires were completed at the morning. The physiological, endocrine, and cognitive performance were then assessed at three steps: immediately before the escape exercise (Baseline), 30 min after the escape exercise (Escape) and two hours after the escape exercise (Recovery). The exercises were supervised by the researcher team so that assessments were controlled at each step of the protocol.

### 2.3 Psychological measures: self-administered questionnaires

The Freiburg Mindfulness Inventory (FMI): Subjects completed the French version of the Freiburg Mindfulness Inventory (FMI). The FMI used in this study was a short form with 14 items developed for common contexts, where knowledge of the Buddhist background of mindfulness cannot be expected [10,17]. This scale derivates from the original FMI which is a thirty-item self-administered questionnaire directly developed qualitatively out of the original Buddhist concept of mindfulness. It constitutes a consistent and reliable scale evaluating several important aspects of mindfulness, which probably is one-dimensional [10]. Each self-descriptive statement was evaluated using a four-point Likkert scale ranging from 1 (strongly disagree) to 4 (strongly agree). Depending on the suggested time-frame, state- and trait-like component could be assessed. In the present study, the short form was used for measuring the mindfulness-trait.

The Spielberger State-Trait-Anxiety Form Y Inventory: The Spielberger State-Trait-Anxiety Inventory (S-STAI) is a 40-item self report questionnaire [18]. In the state portion of the scale, 20 items ask subjects to report the extent of their anxiety at particular moments. In the trait scale, the remaining 20 items ask respondents to indicate the intensity of their anxiety in general. Trait score were used in this study.

## 2.4 Physiological assessments: cardiac vagal tone

The cardiac vagal tone was evaluated from the electrocardiogram (ECG). The EEG was recorded using a portable recorder (Temec instrument, Vitaport CPS) in each experimental step. It was digitalized with a 1000-Hz sampling rate to accurately detect R-wave peaks. The ECG trace and the detection marks were displayed together to be checked. All the ECG data were free from arrhythmia and artifact, except five cases of singular premature heart beats. The time series of interbeat intervals were generated and the spectral analysis of Heart Rate Variability (HRV) was then carried out using the Fast Fourier Transform algorithm [19]. The HRV spectral analysis focused on low- (LF, <0.1 HZ) and high-frequency (HF, >0.15 Hz) bands. The LF rhythm contains both sympathetic and parasympathetic contributions, whereas the HF rhythm primarily reflects vagal efferent tonus. According to published recommendations [20], HRV was quantified by LF/HF, assumed to reflect the cardiac sympathovagal balance.

### 2.5 Endocrine assessments

Salivary cortisol, a reliable marker used for HPA axis activity [4], was sampled at the Baseline, Escape and Recovery steps leading to 39 samples from the 13 subjects. Each 5 mL saliva sample was collected in Salivette tubes according to specification of the provider [Sarstedt; Inc., Newton, NC). Two hours before each collection, eating, drinking or smoking were not allowed. Once filled, the tubes were centrifuged, sampled into 1.5 mL aliquots stored at -80°C until analysis.

The nocturnal urinary free cortisol excretion was assessed as marker of anticipatory anxiety. The subjects were instructed to collect urines from 18h00 to 6h00. Once retrieved, urine volume was measured and a 2-mL sample was taken then stored at -80°C until analysis.



Cortisol concentrations were measured using radioimmunoassay kits according to the protein concentration rates [Siemens Healthcare Diagnostics, Germany). The urinary cortisol excretion rates were calculated according to the diuresis and the creatinine excretion rates.

# 2.6 Mood aand sleep questionnaires

A computerized subjective sleep questionnaire [21] was used to evaluate the quality of the subjects' sleep during the night before each session. It consisted on two items [i.e., restoration quality and easiness to go to sleep) featured by Visual Analogue Scales from "-"on the left to "+" on the right. The subjects "hooked" a visual pointer on a sliding scale using the mouse, and dragged the pointer to the appropriate location on the scale (12 cm long).

The mood was evaluated at the beginning of both sessions using a computerized abbreviated version (22; 5 min) of the Profile of Mood States (POMS). It consisted in an adjective checklist of 37 items rated on a five-point scale that ranges from (1) "not at all" to (5) "extremely". The subjects were asked to answer according their present mood. Six factors were then calculated: anxiety-tension, depression-dejection, anger-hostility, fatigue-inertia, vigor-activity and confusion-bewilderment.

## 2.7 Cognitive test battery

The task battery was built to be fulfilled in less than 15 min, the temporal window allowed by the exercises. It included in the following order. (i) Declarative memory. It was tested using a set of 12 pictures. All the pictures were shown during 10 sec at the beginning of the test battery. The free recall with false recall (recall of pictures outside of the set) and recognition (recognition of pictures of the set among 24 pictures) was assessed 15 min later. A new set of pictures was presented at each time of assessments. (ii) Short-term memory. It was studied using the 10-min Digit span test. A set of numerical digit strings, composed of 4 to 8 digits was presented for learning to the subjects. Each string was presented during 1 sec with a 0.5 s interval between two consecutive strings. The subjects' task was to memorize the complete string of digits and to recall it by clicking using the mouse on the appropriate digits on a visual keypad displayed on the monitor. Recall was required either in the same or the reverse order of presentation. The number and the mean reaction time of correct retrievals were considered.

### 2.8 Data analysis and statistical methods:

The statistical analysis was performed using SPSS 11.0 software package (SPSS Inc., Chicago, IL, USA). Physiological, endocrine and cognitive data time courses (Baseline, Escape and Recovery) were studied using repeated-measure analyses of variance (ANOVA). If necessary, *post-hoc* analyses were carried out using Neuwman-Keuls tests. Relationships among physiological, endocrine, and cognitive responses were studied using Pearson's correlation test. Comparisons between subjects according to their mindfulness level were carried out using independent t tests. Statistical significance was set at p<0.05. Results were expressed as mean and standard deviation.

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### 3.0 RESULTS

# 3.1 Psychological measures: self-administered questionnaires

For the mindfulness scale, the mean score was  $41 \pm 5,32$  with eight subjects above the mean score and five under the mean score. For the Spielberger anxiety scale, results did not differ from the standards observed in a similar population; the mean scores were  $24,77 \pm 6,62$  for the anxiety-state scale and  $27,69 \pm 7,32$  for the anxiety-trait scale. Subjects with higher anxiety-trait score exhibited higher anxiety-state score (r(12)=0.90, p<0.01). When regarding correlations between mindfulness scale and anxiety scales, results showed that submariners prone to higher mindfulness score were prone to lower anxiety-trait score (r(12)=-0.72, p<0.01) and to lower anxiety-state score (r(12)=-0.67, p=0.01).

### 3.2 Effects of the escape training on the physiological, endocrine and cognitive assessments:

Considering physiological responses, no significant difference was observed on LF/HF among the three steps for the group of 13 submariners (Figure 1). No correlation between each step was observed for LF/HF.

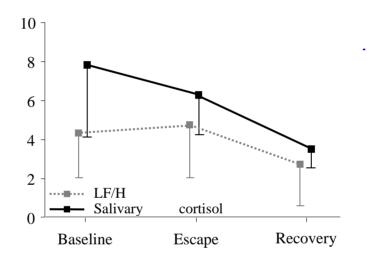


Figure 1 Mean [SD) LF/HF and Mean [SD) salivary cortisol concentrations [nm/L) according to the measurement times in military men during the escape. LF and HF indicate low-frequency and high-frequency, respectively.

When regarding endocrine assessments, salivary cortisol concentrations decreased steadily (Time effect, F(2,12)=6.50, p<0.05; Figure 2) with higher Baseline values compared to Escape (p<0.05) and Recovery (p<0.05) and higher Escape value compared to Recovery (p<0.01). A positive correlation between Escape and Recovery was observed for salivary cortisol (r(12)=0.81, p=0.05). No correlation was observed between cortisol and LF/HF values in either step.

Considering picture recall, no difference was observed among experimental steps.

Considering the Digit-span test, a significant time-effect (F(2, 11)=5, p<0.05) was observed for the number of correct retrieval in case of reverse order of presentation task: Escape (6.16  $\pm$ 1.89) and Recovery (6  $\pm$ 1.86)



were higher than Baseline (5  $\pm 1.76$ , p<0.05 for the two steps). No difference among experimental steps was observed for the direct order task.

## 3.3 Effects of the escape training on the assessments according to the FMI level:

Two groups were defined according to the mean mindfulness score found in the submariners population. The eight subjects with a score above the mean score constituted the FMI+ group and the five subjects with a score under the mean score constituted the FMI- group. Further analyses were carried out between subjects according to their mindfulness level (FMI+ vs. FMI-; figure 2). Considering LF/HF, whether no significant difference was observed between groups for each experimental step, the Escape values appeared lower for the FMI+ than these observed for the FMI- (t(11)=-1.49, p=0.15 with 5.16  $\pm$ 2.56 for FMI+ and 8.38  $\pm$ 5.28 for FMI-). Within each group, no correlation between each step was observed for LF/HF.

Considering endocrine assessments, the nocturnal cortisol excretion rates did not differ between groups. For the salivary cortisol levels, no significant difference was observed between groups for each experimental step. However, the Escape cortisol concentration appeared lower for the FMI+ than these observed for the FMI-(t(11)=-1.34, p=0.17) with 5.28  $\pm 2.75$  nmol/L for FMI+ and 10.00  $\pm 9.51$  nmol/L for FMI-). For the FMI+, positive correlations were observed between Baseline, Escape and Recovery steps (3 correlations ranging 0.71<< 0.86, 0.01 < p < 0.05). This was not observed for the FMI-.

The night before escaping, compare to the FMI- group, the FMI+ groups showed a lower latency to go to sleep (t(11)=2.00, p=0.05 with 5.28  $\pm 2.75$  cm for FMI+ and 9.64  $\pm 1.18$  cm for FMI-) and a higher sleep quality (t(11)=2.18, p=0.05 with 10.32  $\pm 0.83$  cm for FMI+ and 8.38  $\pm 2.34$  cm for FMI-). No difference was observed between groups for the POMS scale, whatever the factor.

No difference between FMI groups was observed for each experimental step when regarding the digit span performances. For the picture test performances, a tendency for FMI+ subjects to have higher correct recall at Baseline than FMI- subjects was observed (t(11)=1.75, p=0.09 with  $9.62\pm1.84$  correct recall for FMI+ and  $7.80\pm1.78$  for FMI-). Moreover, FMI+ subjects exhibited lower false recall than FMI- subjects at Escape (t(11)=-2.12, p=0.05 with 0 false recall for FMI+ and  $0.40\pm0.55$  for FMI-). A similar tendency was observed at Recovery (t(11)=-1.54, t=0.10 with  $t=0.25\pm0.48$  false recall for FMI+ and  $t=0.80\pm0.83$  for FMI-).

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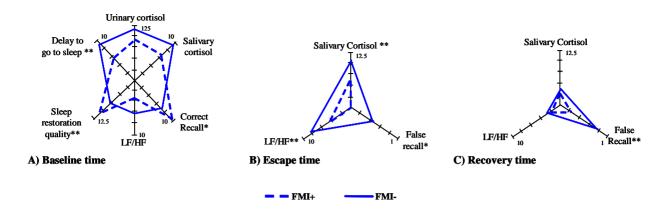


Figure 2: Effects of mindfulness level [FMI+ vs. FMI-) on different assessments according to the times of the escape training. \* p<0.05 for mean differences in responses between the two groups;\*\* p<0.10 for mean differences in responses between the two groups

#### 4.0 DISCUSSION

The present study investigated the physiological and behavioral responses induced by military escape training from a submarine simulator according to the mindfulness-trait level of the submariners. Sympathovagal balance, cortisol salivary levels, and short-term and declarative memory were assessed before (Baseline), 30 min after (Escape) and two hours after (Recovery) the escape exercise. Whereas the sympathovagal response did not differ between the time-points, the endocrine response as shown by the salivary cortisol patterns showed a tendency to decrease during the escape training. Such decrease is congruent with what is observed prior to public speaking or prior to a first jump for novice parachutists [23]. This pattern is highly evocative for an anticipation of threat which disappeared with the beginning of action.

The impact of the escape training on cognitive functions differed according to the assessed performances. For the picture test performances, no difference appeared between the time-points. The pattern of performances of short-term memory for the direct order task also did not differ between the time-points. For the reverse order task, a particular time-pattern was observed, the lower performances being observed for Baseline compared to Escape and Recovery. During the time preceding escaping, submariners had to switch from "remember the procedures for the imminent escape" to "realize the short-term memory task". According to the cognitive load theory [24], the process by which information is maintained or stored on-line for briefs periods of time, is a limited amount of resources available when information is being processed. At Baseline, the resources may not be available due to the reverse order task, known to induce a higher cognitive load compared to the more automatized order task. Conversely, there was no additive operational task during Escape and Recovery, explaining the less impairment in "reverse order" task. This cognitive pattern was concomitant to the saliva cortisol level. Furthermore, it disappeared 2 hours after in parallel with the decrease of cortisol level suggesting that an anticipation of threat also could influence the "reverse order" task performances before the beginning of action.

Lastly, our results highlighted differences in the pattern of responses according with the mindfulness-trait level. At Escape, individuals prone to lower mindfulness score exhibited a higher stress response with a higher



cortisol level which delayed to decrease at Recovery, higher ANS activation and pictures recall degradation. This cognitive degradation was concomitant to the salivary cortisol level. It could be suggested that the mechanisms underpinning such cognitive results involve stress through cortisol elevation. Indeed, glucocorticoids have been shown to influence multiple regions of the brain implied in the memory processes [25,26,4] as the hippocampus involved in the declarative memory (recall ability). Furthermore, the night before escaping, compare to the FMI+ group, they exhibited lower sleep quality suggesting a greater anticipation of threat.

Although the present study had important limitations, i.e. the small size and highly selected sample of subjects, it highlights the impact of mindfulness level differences on conducting operational activities. As notified in the book "in Search of Warrior spirit: teaching awareness disciplines to the Green Berets", Dr Strozzi-Heckler [27] observed that the 25 Green Berets trained six weeks for increasing their mindfulness state improved their skills as soldiers especially in terms of how they viewed themselves in relation to the world around them. Whether such psychological ability also promotes right decisions and ethical behaviours during the heat of a conflict needs further investigations.

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